DEVELOPMENT OF HALF-EGGS OF CHAETOPTERUS PERGAMENTACEUS WITH SPECIAL REFERENCE TO PARTHENOGENETIC MEROGONY

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It has been shown in previous studies on sea urchin eggs (1936, 1938) that the non-nucleate fractions, obtained by centrifugal force, may be activated with parthenogenetic agents and may cleave and develop, without nuclei, to the blastula stage. This I have termed parthenogenetic merogony. Five species of sea urchins have given similar results. I had hoped that the parthenogenetic merogone of another class of animal might develop further than the blastula, and for this reason have made the present study.

The egg of the worm, *Chaetopterus,* fulfills the two requirements necessary for parthenogenetic merogony. First, it can be broken apart readily by centrifugal force so that one can obtain non-nucleate fractions in abundance, and second, the normal egg can easily be made to develop parthenogenetically by treatment with salts. Although my chief concern has been the development of the parthenogenetic merogones, a study has also been made of the fertilized merogones for comparison, as well as of the whole centrifuged egg and the white (nucleate) halves both fertilized and parthenogenetic. Although some of my observations may seem to repeat the earlier ones of Lillie (1906, 1909) and Wilson (1929, 1930), the present study is on fractions of a definite size and known nuclear and cytoplasmic composition whereas previous observations have been made on fragments of irregular size and composition and of assumed nuclear content. Certain differences in my results as compared with previous work may be attributed to this difference in material. Other discrepancies are due to the greater centrifugal force used in these experiments, but particularly to the fact that the eggs have been centrifuged in a medium of cane sugar and sea water. The sugar changes the surface of the egg, and in fact sometimes acts on these eggs as a parthenogenetic agent, similar to KCl. A sugar solution was used as a medium because it can be made, by the addition of the proper amount of sea water, of the same specific gravity as the eggs (as well as, by proper dilution, of the same osmotic pressure), so that the eggs remain suspended during centrifugation, and are free to break into two

halves. It also permits the eggs to orient with the lighter area, containing the polar spindle, toward the centripetal pole of the centrifuge, whereas in sea water alone the heavy eggs are quickly thrown to the bottom of the centrifuge tube and are held in the position in which they are thrown. Although no special study has been made of the relation of polarity of the egg and the cleavage planes to the stratification and plane of breaking caused by the centrifugal force, there seems no doubt from observations with the centrifuge microscope that the eggs in the sugar solution *do* orient with the polar area toward the centripetal axis of the centrifuge. It may be mentioned in passing that the *Arbacia* egg in the 12-cell stage, when the colorless micromeres appear, also orients with the micromeres toward the centripetal pole of the centrifuge; this can be readily observed with the centrifuge microscope. The lack of pigment granules makes these cells lighter than the other cells, which are pigmented. In the *Chaetopterus* egg, the clear polar region of the egg when ready for fertilization (Photograph 18) is lighter than the granular region and therefore the egg orients as stated above. When the eggs are centrifuged immediately on laying, in the germinal vesicle stage (Photographs, 17, 3), there is no visible lighter area, and there is no apparent orientation. This is, I think, in line with Morgan's studies (1937, 1938); he has definitely determined that the germinal vesicle is not eccentric even in eggs still in the parapodia; *it* therefore cannot cause orientation.

Stratification and Breaking

(Photographs 1–16)

The *Chaetopterus* egg stratifies readily. In fact, simply leaving the eggs undisturbed over night in isotonic KCl produces a very nice stratification (Photograph 1). This is true also for the *Arbacia* egg to a slight extent. The *Chaetopterus* egg, both unfertilized and fertilized, stratifies also readily with centrifugal force (Photograph 2). It stratifies similarly to the *Arbacia* egg except that there is no pigment; the stratified egg is almost exactly like that of another sea urchin, *Sphaerechinus granularis.* Some photographs of the stratified eggs of the annelid, *Chaetopterus,* and of the echinid, *Spaerechinus,* are very similar and might easily be mistaken the one for the other except that there is a little more oil in the *Chaetopterus* egg (Cf. Photographs 4 and 5). The unfertilized eggs of *Chaetopterus* were usually in these experiments centrifuged after the polar spindle had formed; it was found difficult to stratify them well (Photograph 3) and almost impossible, with the forces used, to break them apart in the very short interval elapsing

between laying and the breaking of the germinal vesicle. The oil goes to the light pole, then there is a clear layer, then a band of mitochondria which stains purple with methyl green, and then the yellow yolk granules at the heavy pole. (Photographs 4, 6). The nucleus, or rather the first polar spindle, *always* lies in the clear zone under the oil. The eggs are very heavy and are usually thrown to the bottom of the centrifuge tube in the same sugar solution in which *Arbacia* eggs are nicely suspended. The result is that the egg breaks irregularly; usually a small oil cap is thrown off and the rest of the egg breaks into irregular fragments (Photographs 13, 14 of eggs in the centrifuge microscope slide). This is also the case when the eggs are centrifuged in sea water alone. The earlier experiments of Lillie and Wilson and most of Morgan's experiments were done in this way.

It is possible, however, to keep the eggs suspended during centrifugation by using a minimum of sea water with the eggs, and a large amount of the isotonic sugar solution. In this way, after centrifuging

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PLATE I

Stratification and Breaking of Eggs

The photographs were all taken of living eggs with a Leica camera, and for the most part with a water immersion (× 40) lens. The magnification on the plates with the exception of Plate II is approximately 175 ×; Plate II is approximately 250 ×. The smaller photographs were taken with a low power (× 10) lens and are here magnified about 65 ×. The times (i.e., minutes, hours) given are derived from the data recorded at the time of photographing.

PHOTOGRAPH 1. *Chaetopterus* egg kept overnight undisturbed in isotonic KCl.

PHOTOGRAPH 2. Egg centrifuged (after fertilization) for comparison with Photograph 1.

PHOTOGRAPH 3. Egg centrifuged immediately on laying; shows unbroken germinal vesicle; this broke immediately afterwards.

PHOTOGRAPH 4. Stratified *Chaetopterus* egg; oil, clear layer, mitochondria and yolk. Polar spindle is in the clear layer under the oil.

PHOTOGRAPH 5. Stratified egg of *Sphaerechinus granularis* for comparison with Photograph 4; same layers.

PHOTOGRAPHS 6–9. Breaking apart of stratified egg of *Chaetopterus* into two halves of uniform size.

PHOTOGRAPH 10. White half-eggs, containing oil, clear layer, mitochondria and a little yolk; also polar spindle. Note uniformity of size.

PHOTOGRAPH 11. White half-egg showing band of mitochondria; stained with methyl green.

PHOTOGRAPH 12. Yellow half-egg, containing yolk.

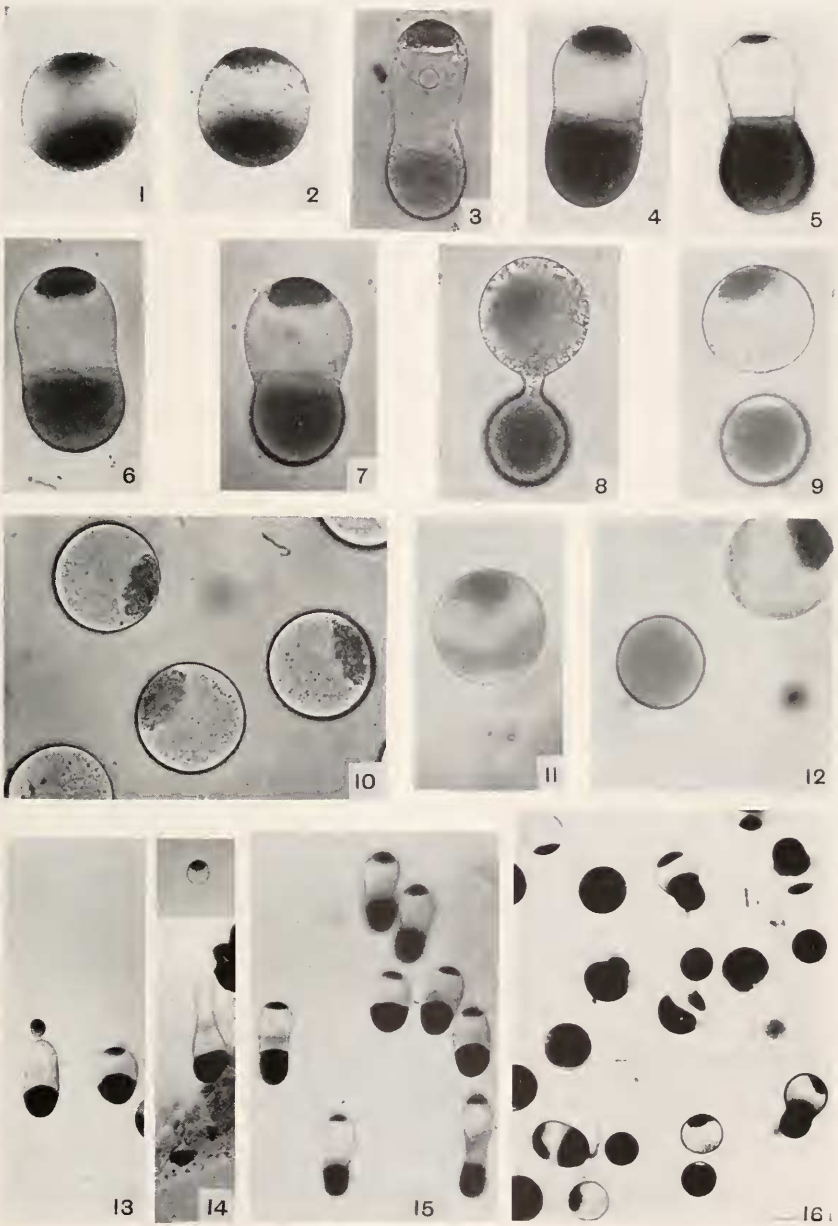
PHOTOGRAPHS 13–15. Eggs immediately after removal from the centrifuge microscope, and still in the centrifuge microscope slide.

PHOTOGRAPHS 13, 14. Eggs supported at bottom of slide. Oil cap pulls off and egg breaks irregularly.

PHOTOGRAPH 15. Eggs suspended in sugar solution. Ready to break into halves of uniform size.

PHOTOGRAPH 16. A group of eggs just removed from the centrifuge tubes, showing regularity of breaking and uniformity of size of halves. The larger black spheres are the whole eggs viewed from above.

PLATE I



Photographs 1–16

for five minutes at 10,000 × g., the eggs stratify, become elongate, then dumbbell shape, and then break into spheres, quite regular in size and quite similar to the halves of the *Arbacia* egg (Photographs 6–9, 16; 15 in the centrifuge microscope slide). The *Chaetopterus* egg breaks, so that the lighter or white half contains oil, clear layer, nucleus (or polar spindle), mitochondria and a little yolk (Photographs 9, 10, 11). The heavier yellow half contains only yolk (Photographs 9, 12). The measurements for the whole and half eggs are:

Whole egg	Diameter	94 μ	Volume	434900 μ^3	
White half	"	83 μ	"	299400 μ^3	436700 μ^3
Yellow half	"	64 μ	"	137300 μ^3	

The white half has over twice the volume of the yellow half. Many batches of eggs have given similar figures.

Normal Development

(Photographs 17–31)

As is well known, especially from the early work of Wilson (1883) and Mead (1895, 1897, 1898 *a*, *b*), the early development of the egg is as follows. The egg is laid in the germinal vesicle stage (Photograph 17); after resting in sea water a few minutes, the germinal vesicle

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PLATE II

Normal Development of the Chaetopterus Egg

Photograph 17. Immature egg immediately on laying; germinal vesicle still intact in center.

Photograph 18. Egg 15 minutes after laying; polar spindle in white area at upper pole.

Photograph 19. Fertilized egg, showing crinkled or fluted fertilization membrane. Twenty-five minutes after fertilization.

Photograph 20. Egg flattened where first polar body is being extruded. Fifteen minutes after fertilization.

Photograph 21. Egg rounded again after first polar body has been extruded; note fluted membrane near polar body. Twenty-five minutes after fertilization.

Photograph 22. Egg flattened again at time of formation of second polar body. Thirty minutes after fertilization.

Photograph 23. Same egg 1 minute later; it has again rounded out.

Photograph 24. Pear-shaped stage. Thirty-four minutes after fertilization.

Photograph 25. Same egg 2 minutes later; beginning of polar lobe.

Photograph 26. Same egg 4 minutes later; 40 minutes after fertilization. First cleavage beginning, with polar lobe still present.

Photograph 27. Same egg 20 minutes later; first cleavage complete and polar lobe withdrawn; note polar bodies near furrow at upper pole.

Photograph 28. Four-cell stage, 1 hour, 40 minutes after fertilization. Note characteristic *Brechungslinie* or cross-furrow.

Photograph 29. Eight-cell stage, 2 hours after fertilization.

Photograph 30. Blastula, just before swimming; 4½ hours after fertilization.

Photograph 31. Trochophore, 1 day after fertilization.


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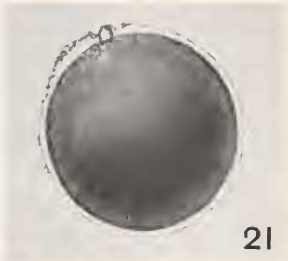
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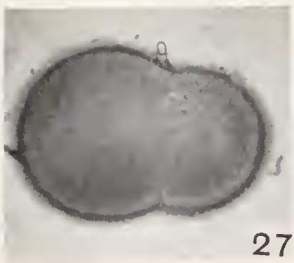
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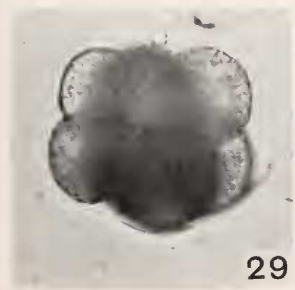
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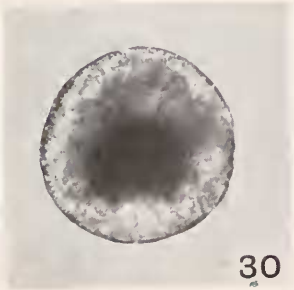
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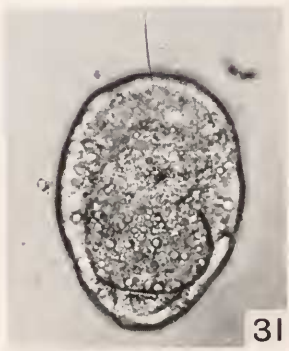
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Photographs 17–31

breaks and the first polar spindle forms. The polar region is seen in the living egg as a clear area near the surface; it is fully formed (at 23° C.) 10–20 minutes after laying (Photograph 18). The egg remains in this condition, with the first polar spindle in the metaphase, indefinitely unless fertilized. If fertilized at this stage, in about 15 minutes[1] the first polar body appears (Photograph 20). The fertilization membrane is the same, according to Whitaker (1933), as the vitelline membrane which lifts from the surface. It is peculiar and characteristic for this egg and quite different from the fertilization membrane of sea urchin eggs. It is crinkled or fluted especially in the region of the polar bodies (Photographs 19, 21). The egg becomes markedly flattened at the pole at the formation of the first polar body (Photograph 20); then becomes spherical (Photograph 21). Then the second polar body is formed, again with a slight flattening (Photograph 22)—about 30 minutes after fertilization; and the egg once again rounds out (Photograph 23). Soon after this (35 minutes after fertilization) the egg becomes pointed or pear-shaped with the narrow end toward the polar bodies (Photograph 24). After again rounding out, the characteristic polar lobe is formed (about 5 minutes after the pear-shape) at the opposite end (Photograph 25). This remains till after first cleavage which begins about 40 minutes after fertilization (Photograph 26). The first cleavage is very unequal giving a small cell and a large one into which the yolk lobe is absorbed (Photograph 27). At the second cleavage, 1½ hours after fertilization, the small cell divides equally and the large cell unequally so that there are two small cells, one large cell and one intermediate in size (Photograph 28), a form of cleavage quite typical for many annelid and mollusk eggs. The next cleavage is shown in Photograph 29, taken 2 hours after fertilization. The spiral type of cleavage which occurs in *Chaetopterus* is difficult to follow in the living egg, so that no attempt has been made to photograph the later cleavage stages of the normal egg, nor to follow them in the experimental work. A normal blastula is shown in Photograph 30, taken just before swimming, which occurs about 5 hours after fertilization; and a one-day trochophore, in Photograph 31.

[1] The times for the various stages are quite variable in the *Chaetopterus* egg even with constant temperature. It differs in this respect from the *Arbacia* egg in which, in the height of the season, the stages occur with almost clock-like regularity at any given temperature. Late in the season (September, October) there is considerable delay in the *Arbacia* egg irrespective of temperature. Eggs may be obtained through November from *Arbacias* which have been brought in before the first part of August and kept in the aquaria in running water. Animals brought in from the sea after August 15 have practically all shed their eggs.

Normal Egg, Parthenogenetic

The parthenogenetic development of the normal uncentrifuged egg by the addition of KCl to the sea water has been described by Mead (1898 *b*), Loeb (1901) and Lillie (1902, 1906), and in detail by Allyn (1912). The peculiar feature is the amoeboid form of the egg and the frequent occurrence of very large nuclei without cell division. The later embryos tend to stick together and form complex multiple organisms as described particularly by Loeb (1901). This is probably due to the effect of the KCl solution on the surface of the cells. Unequal two- and four-cell stages of the characteristic annelid type also occur, and the parthenogenetic egg may give rise to apparently normal swimmers just like the fertilized egg. Some equal two-cell stages also occur.

My best results for parthenogenesis have been obtained by putting the eggs into a solution of 1 gram KCl + 100 cc. of sea water for 30 minutes, then transferring them to sea water. However, I have found other proportions and times of exposure to work better with some batches of eggs. The different batches respond quite differently to the KCl solutions and I could get no dependable standard solution.

Whole Egg Centrifuged, Then Fertilized

(Photographs 32–42)

The *centrifuged* whole egg, which has been elongated by the centrifugal force, becomes spherical again quite quickly after the force is removed, and this whether the egg is fertilized or not. It is thus strikingly different from the sea urchin egg which becomes spherical if left unfertilized, but retains its elongate or dumb-bell shape if fertilized or artificially activated, all through the cleavages up to the swimming blastula. After fertilization of the centrifuged egg, in *Chaetopterus,* the polar bodies usually come off near, but not on top of, the oil cap (Photograph 32); if the oil cap has been centrifuged off, however, they usually come off exactly at the centripetal pole (Photograph 33); the oil cap apparently interferes with the protrusion of the polar bodies. This is shown also by the results of centrifuging the egg immediately on laying, while the germinal vesicle is still intact, as shown in Photograph 3. When, after removal from the centrifuge, the germinal vesicle breaks, it dissipates the oil in clumps (Photograph 34). The polar spindle forces itself to the center of the oil, which now forms a circle of clumps around it (Photograph 35). After fertilization, the polar bodies come off in the center of the ring of oil clumps.

The fertilized centrifuged egg usually passes through the changes in shape characteristic of the normal fertilized egg as described above, in-

cluding the formation of the polar lobe. It often divides unequally and typically in the first cleavage (Photograph 36), and the second cleavage as well as later ones (Photograph 39) may be of the characteristic annelid type as has been described by Lillie (1906) and Wilson (1929). Some of the cleavages, however, especially in strongly centrifuged eggs, are irregular and atypical. The first division plane is usually perpendicular to the stratification (Photograph 36). It may, however, pass parallel with the stratification, separating the egg into a clear and a granular zone (Photograph 37). These zones may develop separately just as in the centrifuged *Arbacia* egg (Harvey, 1932); the upper clear area usually divides into cells but the lower yolk half is characterized by nuclear without cytoplasmic divisions (Photograph 38). Many swimming, apparently normal, larvae have arisen from the fertilized centrifuged eggs (Photograph 40). But the blastulae tend to gather together in groups and fuse with each other and with the white halves,

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PLATE III

PHOTOGRAPHS 32–42. *Eggs centrifuged, then fertilized.* Photographs 43–46. *Eggs centrifuged then treated with KCl; parthenogenetic.*

PHOTOGRAPH 32. Polar bodies at side of oil cap; 23 minutes after fertilization.

PHOTOGRAPH 33. Polar bodies at centripetal pole where oil cap has been centrifuged off. Twenty-five minutes after fertilization.

PHOTOGRAPH 34. Egg was centrifuged immediately on laying (see Photograph 3); germinal vesicle breaks after removal from centrifuge and scatters oil in clumps.

PHOTOGRAPH 35. On formation of polar spindle a few minutes later, the clumps of oil are forced into a ring.

PHOTOGRAPH 36. Normal cleavage of centrifuged egg; cleavage plane at right angles to stratification. Note polar lobe. Fifty minutes after fertilization.

PHOTOGRAPH 37. Cleavage plane parallel with stratification. One and one-quarter hours after fertilization.

PHOTOGRAPH 38. Later stage of Photograph 37; upper cells have cleaved several times, lower cell has not cleaved, but the nuclei have divided. One and three-quarter hours after fertilization.

PHOTOGRAPH 39. Normal development of centrifuged egg. Two and one-half hours after fertilization.

PHOTOGRAPH 40. Same, four and three-quarter hours after fertilization; just before swimming.

PHOTOGRAPH 41. Fusion of whole centrifuged and white half-blastulae. Seven hours after fertilization.

PHOTOGRAPH 42. A pair of these, high power.

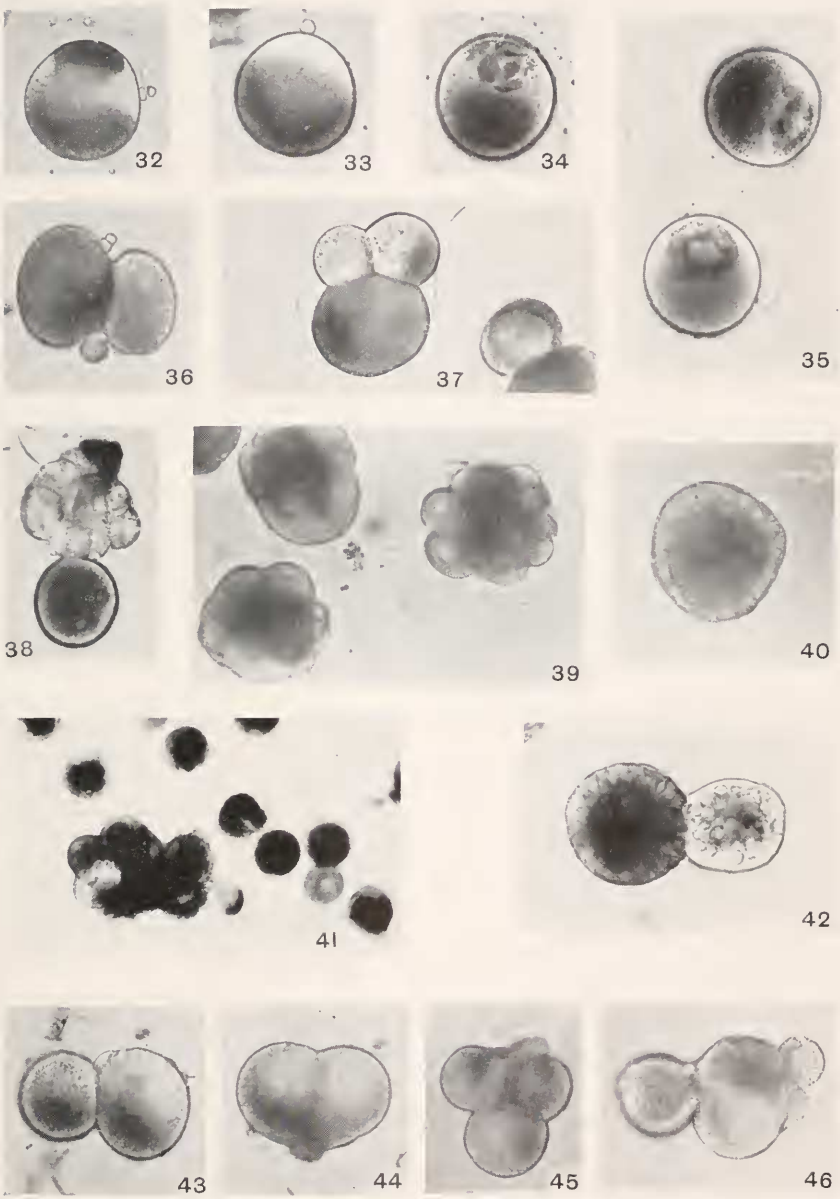
PHOTOGRAPH 43. Two-cell stage of parthenogenetic centrifuged egg; typical unequal blastomeres; cleavage plane perpendicular to stratification. Two hours after treatment.

PHOTOGRAPH 44. Same, equal cells. Note polar lobe.

PHOTOGRAPH 45. Four-cell stage, parthenogenetic. Three hours after treatment. Compare with Photograph 74, of the yellow half-egg fertilized.

PHOTOGRAPH 46. Amoeboid form of parthenogenetic centrifuged egg, 5 hours after treatment. Compare with parthenogenetic white half, Photograph 58; with fertilized yellow half, Photograph 75; and with parthenogenetic merogone, Photograph 96.

PLATE III



Photographs 32–46

forming complex organisms (Photographs 41, 42) just as is characteristic of the parthenogenetic normal eggs. This agglutination must be due to the effect of the sugar solution (in which the eggs have been centrifuged) on the surface of the eggs. It cannot be due entirely to the lack of a fertilization membrane, since eggs from which fertilization membranes have been removed by shaking, and which were kept in sea water, without sugar, do *not* agglutinate.

Centrifuged Egg, Parthenogenetic

(Photographs 43–46)

The centrifuged egg will develop parthenogenetically by the addition of KCl to the sea water, in exactly the same way as the uncentrifuged. Polar bodies may be given off often while still in the KCl solution, and there occur some typical normal cleavages (Photograph 43). But there is a tendency toward equal first cleavage (Photograph 44) and most of the eggs soon become amoeboid and develop with large nuclei and few cell divisions like the uncentrifuged parthenogenetic eggs (Photographs 45, 46). The blastulae tend to fuse together just as do the parthenogenetic normal eggs and the fertilized centrifuged eggs. Development is slower in the parthenogenetic eggs, and swimmers occur in 8 hours instead of 5. This has been found true also of parthenogenetic normal eggs of *Chaetopterus* as well as of many other eggs.

White Half-egg, Fertilized

(Photographs 47–55)

The white half-egg, which contains the polar spindle, can be fertilized, and may develop exactly like the normal whole egg. The egg passes through the flattened and pear-shape phases and the polar bodies are usually given off near the oil cap; these are usually larger than in the normal egg (Photograph 47). The egg usually divides unequally in the first cleavage (Photograph 48, lower egg) and the large cell unequally in the second cleavage giving the typical four-cell stage of two small cells, one large and one intermediate in size (Photograph 49, lower egg). The nuclei are especially striking owing to the lack of granules; these obscure the nuclei in the living whole eggs. There is a tendency for the egg to become amoeboid even before the first division, so that it is not possible to determine if there is a polar lobe or not (Photograph 50). There are often protuberances from one of the blastomeres resembling polar lobes and these occur also in the four-cell stage (Photograph 51). There is also a tendency toward equal first cleavage. Normal blastulae and swimming trochophores are often

formed exactly like those of the whole egg, except that they are white instead of yellow and are smaller (Photograph 52, inset, upper left). But frequently, the blastulae have irregular sized cells and large nuclei like the parthenogenetic whole eggs and they tend to become amoeboid (Photograph 52). The blastula shown in Photograph 53 was both amoeboid and ciliate. These white blastulae often stick together in clumps and fuse with each other and with the whole blastulae present in the same lot (Photographs 54, 55, 41, 42). As pointed out above, this is probably due to the effect of the sugar solution on the surface. The development of the fertilized white halves takes place at about the same rate as the whole eggs; swimmers occur in 5 hours.

The striking thing is that the white half-eggs often cleave, at least in the early stages, exactly like the whole eggs in the very peculiar and characteristic pattern typical of the annelid egg. This has been found also to be the case in many irregular fragments studied by Lillie (1909) and Wilson (1929).

White Half-egg, Parthenogenetic

(Photographs 56–62)

The white half-eggs may be activated by KCl similarly to the whole eggs; some have been activated and have produced swimmers without further treatment than the sugar solution in which they were centrifuged. They may throw off polar bodies often while still in the KCl solution (Photograph 56) and may cleave in the typical annelid fashion (Photograph 57), at least to the four-cell stage. But they, like the whole parthenogenetic eggs, often divide equally at first cleavage, and they usually become amoeboid and develop with irregular cells and large nuclei (Photographs 58–62); these irregularities are much more pronounced in the parthenogenetic than in the fertilized white halves. Development is also delayed as is usual with parthenogenetic eggs.

Yellow Half-egg, Fertilized (Fertilized Merogone)

(Photographs 63–84)

The yellow half-egg, which contains no nucleus, may be fertilized and the fertilization membrane is lifted off. I have observed no polar bodies of which I could be sure, though there are often clear amoeboid protrusions simulating polar bodies. One would not, of course, expect polar bodies in this half-egg, since the polar spindle is always in the other half. There is also no polar lobe of which one can be sure. There is usually no cleavage of this half-egg, but it becomes amoeboid and may live in this condition for several days. In some eggs, I have

found, several hours after fertilization, a nucleus, from the sperm, and later two nuclei, and much later several nuclei of different sizes—that is, nuclear division without cell division (Photographs 63–65), such as is characteristic of the heavy red halves of the *Arbacia* egg (Harvey, 1932). However, several eggs have given a typical first cleavage of two unequal cells (Photographs 66, 67). Several eggs have divided into two equal cells, and in one case these divided again equally giving four equal cells—not typical for this form (Photographs 68, 69). These four cells, later on, fused, cleaved again, became amoeboid and soon went to pieces (Photographs 70–75). In general a failure to cleave and a tendency to become amoeboid are characteristic of the fertilized yellow halves or fertilized merogones. No blastulae or ciliated structures from the fertilized yellow halves have been observed. A series of photographs (76–84) will show the extreme amoeboid activity of one of these eggs in the two-cell stage. These photographs

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PLATE IV

White half-eggs, fertilized (47–55) *and parthenogenetic* (56–62)

PHOTOGRAPH 47. White half fertilized, with polar bodies. Twenty-five minutes after fertilization.

PHOTOGRAPH 48 (lower egg). Typical two-cell stage. One and one-quarter hours after fertilization.

PHOTOGRAPH 49 (lower egg). Typical four-cell stage. One and one-half hours after fertilization. Note *Brechungslinie* or cross-furrow as in Photograph 28. In upper egg, all the oil is in one blastomere, and the blastomeres are not quite typical in size.

PHOTOGRAPH 50. Just before first cleavage, amoeboid at position for polar lobe. Fifty minutes after fertilization.

PHOTOGRAPH 51. Four-cell, amoeboid. One hour after fertilization.

PHOTOGRAPH 52. Amoeboid blastulae with large irregular nuclei, and also normal white blastula (inset, upper left). At the upper right is a normal whole blastula (centrifuged). Five hours after fertilization.

PHOTOGRAPH 53. White blastula, amoeboid and also ciliated. Six and one-half hours after fertilization.

PHOTOGRAPH 54. White blastulae fused together. See also Photograph 41. Six hours after fertilization.

PHOTOGRAPH 55. A pair of fused blastulae, high power. See also Photograph 42.

PHOTOGRAPH 56. Parthenogenetic white half with two polar bodies. One hour after activation.

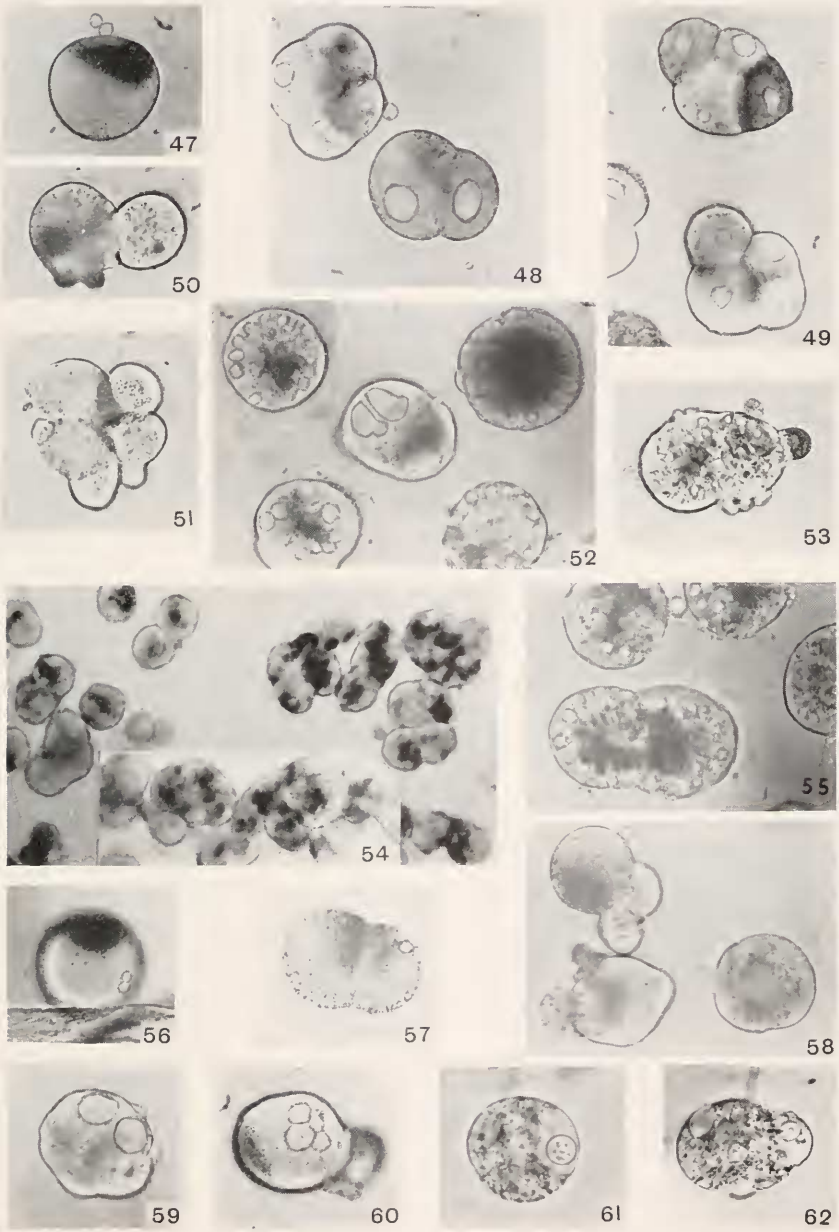
PHOTOGRAPH 57. Typical two-cell stage of parthenogenetic white half. Two hours after activation.

PHOTOGRAPH 58. Parthenogenetic white halves, amoeboid. One and one-half hours after activation. Cf. with Photograph 46 of whole centrifuged egg, parthenogenetic.

PHOTOGRAPHS 59, 60. Parthenogenetic white half blastulae, amoeboid, with irregular nuclei. Four hours after activation. Cf. with Photograph 52, fertilized white halves.

PHOTOGRAPHS 61, 62. Blastulae both ciliate and amoeboid. Twenty-four hours after activation. Cf. Photograph 62 with Photograph 53, fertilized white half.

PLATE IV



Photographs 47–62

were taken at short intervals; after two hours the egg ended up in a two-cell stage, much as it was in the beginning. The meaning of this is not known, but perhaps it points to a similar condition, with regard to the cell surface, for amoeboid motion and cell division.

Yellow Half-egg, Parthenogenetic (Parthenogenetic Merogone)

(Photographs 85–96)

The yellow half-eggs, containing no nucleus or polar spindle, may be activated artificially by KCl. These are the parthenogenetic merogones. After activation, a thick fluted membrane is lifted off exactly like that of the normal fertilized egg, and quite characteristic of this

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PLATE V

Yellow half-eggs, fertilized. (Fertilized merogones)

Photograph 63. Sperm nucleus in yellow half-egg. Two hours after fertilization.

Photograph 64. Same egg with two nuclei, a few minutes later.

Photograph 65. Another yellow half-egg with several nuclei but no cell division. Five hours after fertilization.

Photograph 66. Two-cell stage; cells unequal.

Photograph 67. Typical two-cell stage. Two hours after fertilization.

Photographs 68–75. Successive photographs of the same egg showing cleavage and fusion of blastomeres, and amoeboid character.

Photograph 68. Two equal cells. Two hours after fertilization.

Photograph 69. Five minutes later, 4 equal cells.

Photograph 70. Ten minutes later. Blastomeres begin to fuse.

Photograph 71. Three minutes later. Possibly polar lobe.

Photograph 72. One minute later. Three cells; two have fused.

Photograph 73. Six minutes later. Four (or 5) separate cells again.

Photograph 74. Five minutes later. Fusion of blastomeres, and egg becoming amoeboid. Compare with Photograph 45, of the whole centrifuged egg, parthenogenetic.

Photograph 75. One-half hour later, 3 hours after fertilization. Amoeboid. Compare with Photograph 46, of whole centrifuged egg, parthenogenetic; and with Photograph 58 of parthenogenetic white half and with Photograph 96 of parthenogenetic merogone.

Photographs 76–84. Successive photographs at short intervals showing amoeboid character of a two-cell stage of the fertilized yellow half-egg.

Photograph 76. Two-cell stage, almost equal blastomeres. Fertilized at 9.30 A.M., photographed at 10.50 A.M.

Photograph 77, at 11.35 A.M.

Photograph 78, at 11.40 A.M.

Photograph 79, at 11.50 A.M.

Photograph 80, at 12 noon.

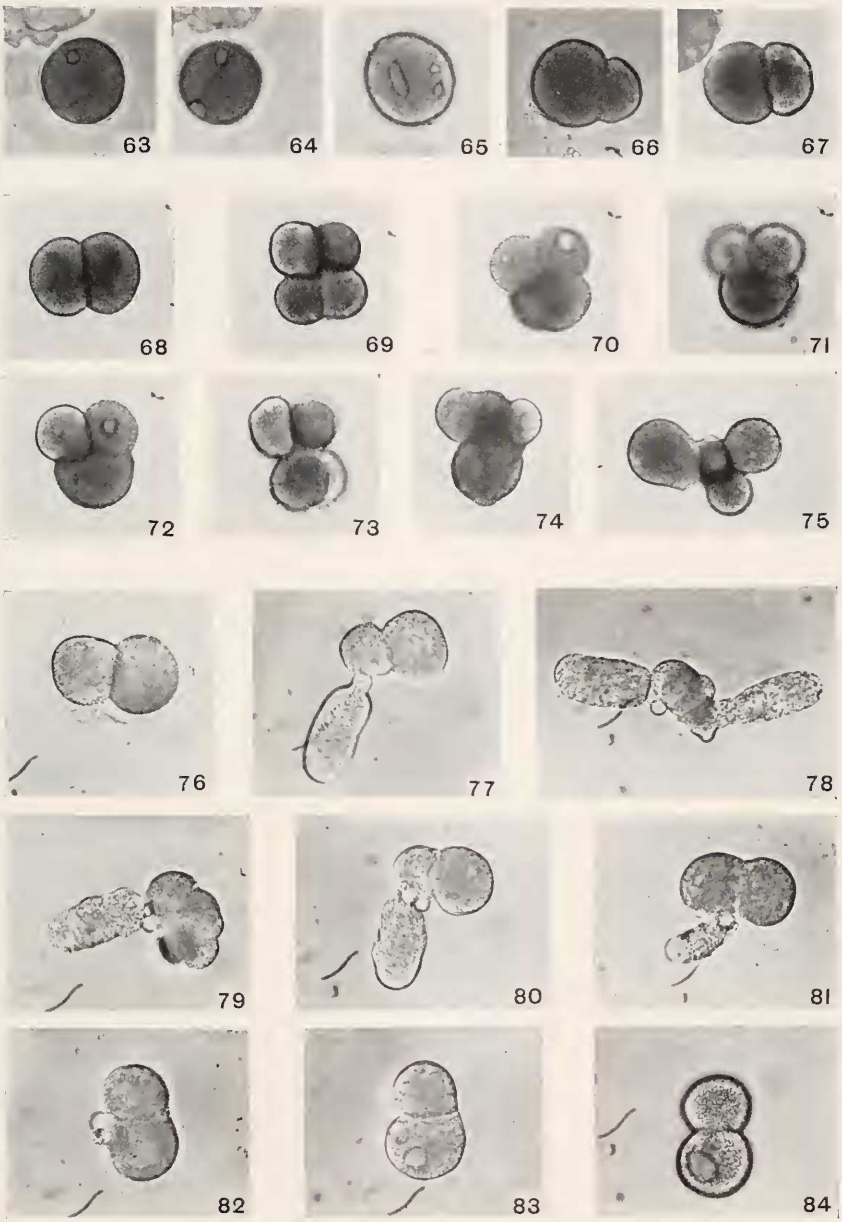
Photograph 81, at 12.10 P.M.

Photograph 82, at 12.20 P.M.

Photograph 83, at 12.21 P.M., two-cell stage, slightly unequal.

Photograph 84, at 1.10 P.M., two cells almost equal and quite similar to the original, Photograph 76. Note large nucleus.

PLATE V



Photographs 63–84

particular egg (Photograph 85, cf. Photographs 19, 21). They then usually become amoeboid and develop no further, though they may live for several days. There is no definite polar lobe, but amoeboid processes sometimes simulate it. Some cases of first cleavage have been observed, however, and these were of both the equal type and the unequal type with varying degrees of inequality (Photographs 86–90); these also became amoeboid and there was no further development (Photographs 91–93). The amoeboid activity of the parthenogenetic merogone is shown in a series of photographs taken at intervals of a few minutes (Photographs 94–96). The parthenogenetic merogone, then, resembles the fertilized merogone in its tendency to become amoeboid and in its limited cleavage, though the fertilized merogone goes perhaps one step further in development. (Compare Photographs 86–96 with Photographs 66–84).

Discussion

A study of the *Chaetopterus* egg has shown that the non-nucleate fraction of the egg of another type of animal than the sea urchin can be activated artificially. The parthenogenetic merogone of *Chaetopterus*

PLATE VI

Yellow half-eggs, parthenogenetic. (Parthenogenetic merogones)

Photograph 85. Yellow half, parthenogenetic, with characteristic fluted fertilization membrane. One hour after activation. At right upper corner is part of a whole cell, also with fluted membrane. Compare also with Photograph 19, of the normal whole egg.

Photograph 86. Two-cell stage, unequal. Two hours after activation. Compare with Photograph 66, of fertilized merogone.

Photograph 87. Typical two-cell stage. Two hours after activation. Compare with Photograph 67, of fertilized merogone.

Photograph 88. Cleaving into two almost equal cells. Five hours after activation.

Photograph 89. Two equal cells. Six hours after activation. Compare with Photograph 68 of fertilized merogone.

Photograph 90. Two equal cells. Five hours after activation. Compare with Photograph 76. Clear protrusion looks like a polar body.

Photographs 91, 92, 93. Amoeboid forms of older parthenogenetic merogones. Twenty-four hours after activation. Compare with Photograph 46 of whole centrifuged egg, parthenogenetic, and with Photograph 75, of fertilized merogone.

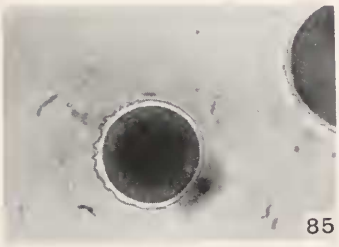
Photographs 94–96. Successive photographs of the same parthenogenetic yellow half-egg, taken at very short intervals to show amoeboid character. Eighteen hours after activation. Compare with Photographs 76–84 of the fertilized merogone. Also compare Photograph 96 with Photograph 75 of the fertilized merogone, and with Photograph 46 of the parthenogenetic whole egg, and with Photograph 58 of the parthenogenetic white half.

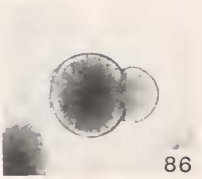
Photograph 94. Activated at 5 P.M. July 21, photographed at 11 A.M., July 22.

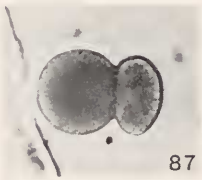
Photograph 95. At 11.02 A.M.

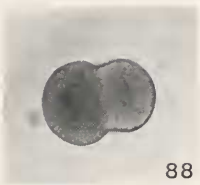
Photograph 96. At 11.03 A.M.

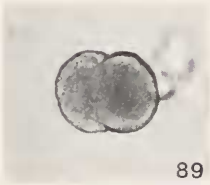
PLATE VI

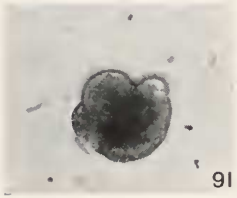


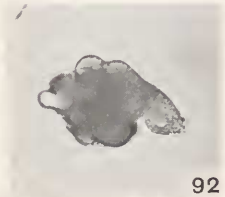


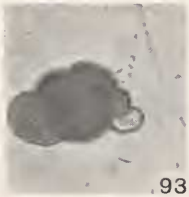


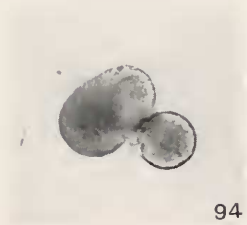


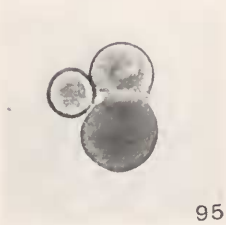


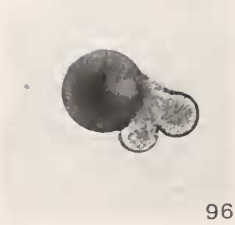












Photographs 85–96

goes through only one cleavage and thus does not develop as far as that of the sea urchin. This is not due, however, to the lack of a nucleus, since the same fraction fertilized does not develop much further. The unfertilized *Chaetopterus* egg is perhaps more highly organized than the unfertilized sea urchin egg.

The parthenogenetic development of the normal *Chaetopterus* egg is peculiar in that usually there is no orderly cleavage with the accompanying nuclear divisions, but instead, amoeboid forms with irregular cells and large nuclei. It is not surprising, therefore, that there is no orderly development of the parthenogenetic merogone, and that it likewise, becomes amoeboid. It may be that the concentration of heavy granules into this half of the egg is a factor also in preventing further development. This is suggested by the experiments of Whitaker and Morgan (1930), who obtained polar and anti-polar halves of the *Chaetopterus* egg by cutting with a needle. These halves have, of course, a more even distribution of granules than the halves obtained by centrifuging. They found that after fertilization, both of the halves would cleave unequally like the whole egg, and that the anti-polar half would form a polar lobe; there were certain irregularities, however. But the anti-polar half obtained by cutting develops more normally, at least in the early stages, than the anti-polar half obtained by centrifuging. It is of interest, too, that the parthenogenetic merogone of the *Sphaerechinus granularis* egg, whose stratification is so similar to that of *Chaetopterus* (Photographs 4 and 5) also becomes amoeboid and does not cleave regularly; the parthenogenetic centrifuged whole egg of this form likewise becomes amoeboid (see Photographs 53–56 of my 1938 paper).

The amoeboid activity which is characteristic of the parthenogenetic egg or its fraction both in *Chaetopterus* and *Sphaerechinus*, may be largely due to the action of the parthenogenetic agent on the surface of the egg. Amoeboid activity may be another expression of the condition of the cell surface characteristic of cleavage just as gelation seems to be characteristic of some of the interior cytoplasm at this time, in the formation of asters.

That the early development is dependent on the cytoplasm rather than the nucleus is brought out again in the development of the white halves of the *Chaetopterus* egg. These, whether fertilized or parthenogenetic, may cleave according to the peculiar and characteristic annelid pattern. It seems remarkable that these fractions, and even very much smaller fragments, according to Lillie and Wilson, may cleave just like the whole egg. This certainly leads one to the conclusion that it is the matrix or clear substance in the egg, rather than nuclei or visible granules that is important in early development.

Attention may be called to the frequent occurrence of equal cells in the first cleavage instead of the typical unequal cells. In the present experiments, there is a tendency to equal division in parthenogenetic whole eggs, both normal and centrifuged and both in the white and yellow halves both fertilized and parthenogenetic. Other observers have noted the same thing in certain of these cases. In the *Cumingia* egg, I found that pressure on the egg after fertilization had the same effect (Browne, 1910) and similar results have been obtained by Tyler (1930) for the *Chaetopterus* egg; he found also that cold, heat, ultra-violet rays and anaerobiosis would produce the same effect. One would assume that an unequal division of the two cells is a more specialized form, and that whatever mechanism is responsible for it, is put out of gear by certain experimental conditions. It seems to be due, at least in many cases, to an action especially on the surface of the cell, rather than on the interior.

Summary

1. The unfertilized *Chaetopterus* egg (94 μ) may be stratified and broken into unequal halves of a uniform size by centrifugal force. The nucleate white halves (83 μ) contain oil, clear layer, mitochondria and a little yolk. The non-nucleate yellow halves (64 μ) contain only yolk.

2. The centrifuged whole egg may develop, both fertilized and parthenogenetic, similarly to the normal uncentrifuged egg. Certain peculiarities have been noted.

3. The white halves, both fertilized and parthenogenetic, may cleave according to the typical annelid pattern. But there is a tendency toward equal first cleavage and amoeboid form and later, development with irregular cells and large nuclei; the blastulae tend to fuse together; these peculiarities are much more pronounced in the parthenogenetic than in the fertilized white halves.

4. The yellow halves, both fertilized (= fertilized merogone) and parthenogenetic (= parthenogenetic merogone) usually lift off a characteristic fluted fertilization membrane and pass through only one cleavage and become markedly amoeboid.

5. Early development without nuclei (parthenogenetic merogony) is established for the annelid, *Chaetopterus,* in addition to the five species of echinoderms previously studied. The development of the parthenogenetic merogones of *Chaetopterus* does not go as far as that of the sea urchin.

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